

# Species

## Seasonal Variation of Airborne Microflora in Dairy Processing Plant

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### ABSTRACT

A study was conducted to estimate airborne bacterial and fungi load in dairy processing plant and its surroundings in different seasons of the year. Air samples were taken in different seasons of the year including winter, summer and monsoon to know the air borne microbial load in morning, afternoon, evening, night and midnight period. The highest bacterial and fungal population was recorded in the evening during 18 – 20 hrs compared to morning, afternoon, night and midnight hours in each season. It was also observed that during monsoon season. During rain, there was a drastic reduction in bacterial and fungal population. The bacterial isolates were characterized and identified by Gram staining. Among the total bacterial isolates, they were contributed to Gram positive cocci (68%), Gram positive *Bacilli* (12%), Gram negative short rods (8%), and Gram positive filamentous *Actinomycetes* (12%). Fungal isolates were characterized by gross colony appearance and by microscopic observations. Fungal isolates includes *Mucor* (32%), *Penicillium* (27%) *Rhizopus* (18%), *Aspergillus* (12%), *Fusarium* (3%), *Neurospora* (2%), *Alternaria* (2%), *Cylindrocarpon* (3%) and *Helminthosporium* (1%) within dairy processing plant.

**Keywords:** Dairy processing plant, Airborne microflora, Seasons of the year, Air convection, Total bacterial population, Total fungal population.

**Abbreviation:** hr- Hours

### 1. INTRODUCTION

Microorganisms present in the atmosphere may be free or adhered on dust as bacteria, fungi, lichen, protozoa, viruses and algal cell. The airborne contamination load depends upon several factors such as metrological conditions of the locality, sun light, altitude above the ground level, day/night period of air currents, temperature and humidity of the air etc. (Shah et al. 1993). The composition and contribution and concentration of these particles are also related to human activities and climatic condition (Lacey et al. 1981). Atmospheric pollution is one of the most pressing problems. This pollution has now increased to advance level which threat to the health (Jaffal et.al. 1997). Microorganisms are the primary source of indoor air contamination. The indoor air environment can affect any food product which is being manufactured at plant level at greater risk than the outside environment, because enclosed space has confined aerosols increases higher microbial population in food manufacturing plants (Lewis et al. 1994). Indoor biological pollution is recently receiving the afforded attention. The apparent lack of interest of clean air may lead to the difficulties and thus needs to be taken care in the evaluation of their variable effects on food products (Glick et al. 1978). The atmosphere having favourable conditions enhances or promotes the survival of microorganisms in the air. The air is composed of 75% nitrogen, 21% oxygen, 0.9% argon, 0.03% carbon dioxide and 0.076% other trace gases by volume, very low concentration of organic and inorganic nutrients and free water (Lacey et al. 1981). Indoor air of dairy processing plant contains a diverse range of microbial

population. The significance of these microbes is detectable. The importance of estimation of the quantity and type of air borne microorganisms is to use it as an index for the relative hygienic condition of the environment.

The source and spread of organisms inside the dairy processing plant are the important issues. Human related organisms found in clothing are spreaded through human activities in processing plant. The organisms which are spreaded may include *Staphylococcus aureus*, *Micrococcus* spp. alpha hemolytic *Streptococci* and Gram negative short rods. Environmental organisms such as *Bacillus* spp. *Streptomyces* spp. and other bacteria are added from different sources such as air dust, soil and water. The microbial population in a given indoor environment is influenced by many factors including regional climatic condition, percentage of humidity in the air, temperature of working area, the number of visitors, the amount of materials brought in from outside, etc.

Indoor air can be potential source for microbes inside the dairy processing plant. For this reason, knowledge of the incidence of microflora inside dairy products manufacturing unit is important for understanding of the air borne contamination. Controlling the microbes in dairy processing indoors may help in prevention of contamination of dairy products.

### 2. SCOPE OF THE STUDY

The aim of carrying out this research is to find out the air convection effect on bacterial and fungal microflora present in the air during morning, afternoon, evening and night times. The aim also include together comparative data of

Table 1 Air borne microbial Population during winter season

Period	Within Processing Plant		When there was no Production		Outside the Processing Plant	
	During Production					
	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)
Morning	17	18	10	12	39	45
Afternoon	11	12	7	9	29	36
Evening	13	19	13	17	44	49
Night	4	5	4	6	30	21
Midnight	3	6	5	5	25	7

Table 2 Air borne microbial Population during summer season

Period	Within Processing Plant.				Outside the Processing Plant.	
	During Production		When there was no Production		Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)
	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)		
Morning	21	16	13	6	47	39
Afternoon	17	11	6	5	36	25
Evening	24	16	15	9	21	29
Night	9	6	4	4	24	31
Midnight	4	3	4	3	16	15

Table 3 Air borne microbial Population during monsoon season

Period	Within Processing Plant.				Outside the Processing Plant.	
	During Production		When there was no Production		Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)
	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)	Total Bacterial Population (cfu/15 Min.)	Total Fungal Population (cfu/15 Min.)		
Morning	18	21	13	10	21	26
Afternoon	11	14	7	6	15	11
Evening	21	25	11	13	31	27
Night	14	12	5	4	11	7
Midnight	7	6	2	1	6	9

microbes present in the air in different seasons include winter, summer and monsoon. The scope comprises to study out contribution of different variants and their types present in the air.

## 2.1. Materials

The materials used include bio chemical media for to conduct out the study and mounting media for microscopic examination includes;

- Plate Count Agar, Himedia (M091A): For cultivation purpose of Bacteria.
- Potato Dextrose Agar, Himedia (M096): For cultivation purpose of Fungi.
- Immersion Oil, Himedia (RM225): As a mounting media for microscopic examination of Bacteria
- Lacto phenol Cotton Blue, Himedia (S016): As a mounting media for microscopic examination of Fungi.
- Gram Stains-Kit, Himedia (K 001): For Bacterial Staining.

## 2.2. Methodology

In the study, location specific indoor and outdoor microflora has been studied. The present study was aimed at investigating the quantity and quality of air borne microorganisms inside and outside the dairy processing plant.

### 2.2.1. Study Area

Indoor area of a milk processing plant having handling capacity of about one lakh liters of milk per day and it's surrounding is considered for the study. The air samples for the study were taken in different seasons of the year and at different times including morning 8 -10 hrs, afternoon 12 - 14 hrs, evening 18 - 20 hrs, night 21 - 23:30 hrs and at midnight at 24 hrs. The Samples were collected during production and without production period. The samples were also taken from surrounding of dairy processing plant during the same time intervals to check microbial population.

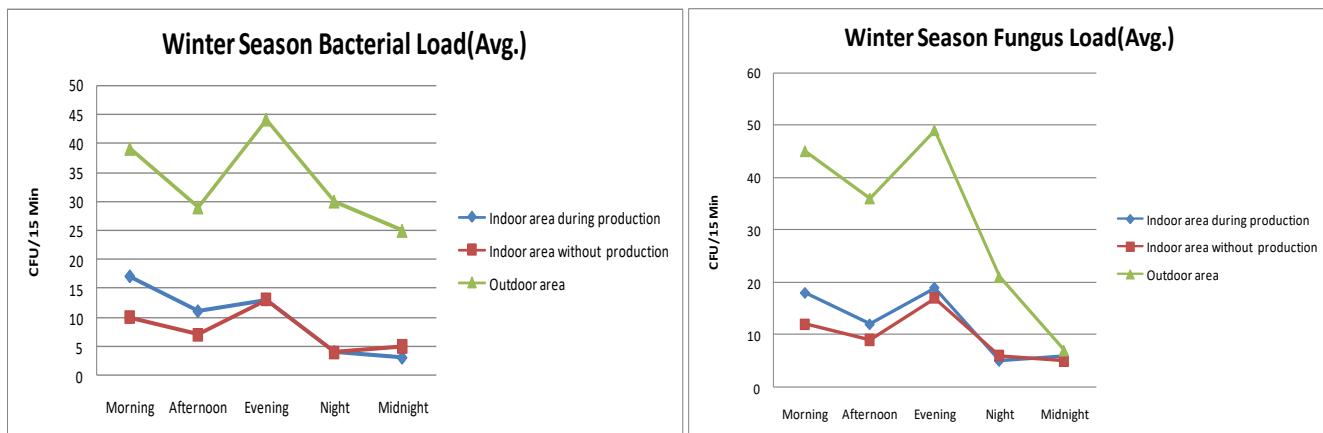
### 2.2.2. Air Sampling and Microbiological examination

The air samples were collected using settling plate technique, by exposing the Petri dishes containing Standard

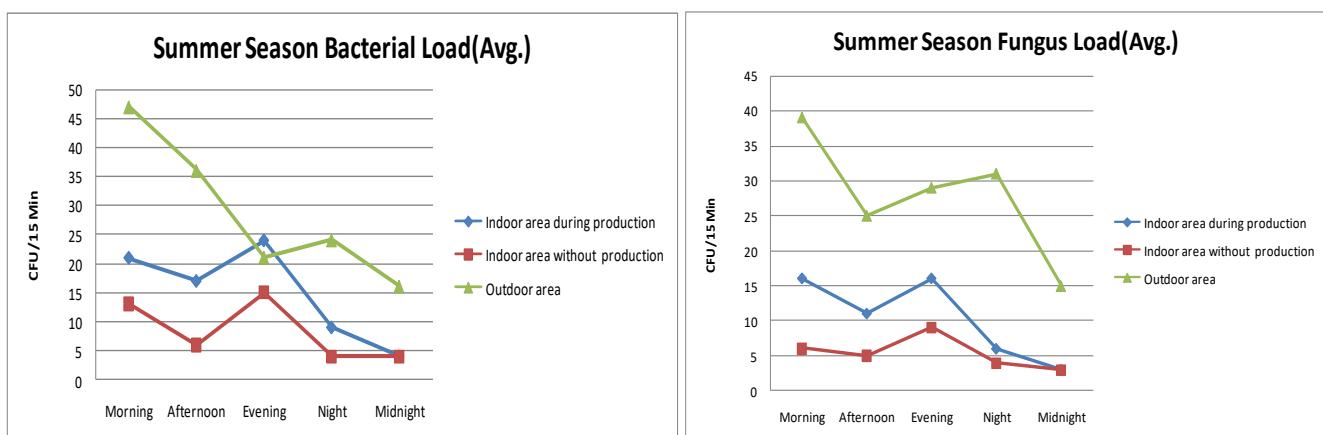
Plate Agar and Potato Dextrose Agar for a period of 15 minutes. After exposure, the plates were incubated for examination. The bacteria cultures were incubated at 37° C for 48 hours, while fungal culture plates were incubated at 25° C for 5 days. To know the amount of microbial load in air of dairy processing plant, every time set of three plates were exposed and its average reading is reported. Bacterial colonies were initially characterized by morphology and identified further by microscopic examination using Grams Reagent kit. The fungal colonies were identified according to its morphology, gross colonial appearance and microscopic examination using lacto phenol cotton blue as a mounting medium.

## 3. RESULTS

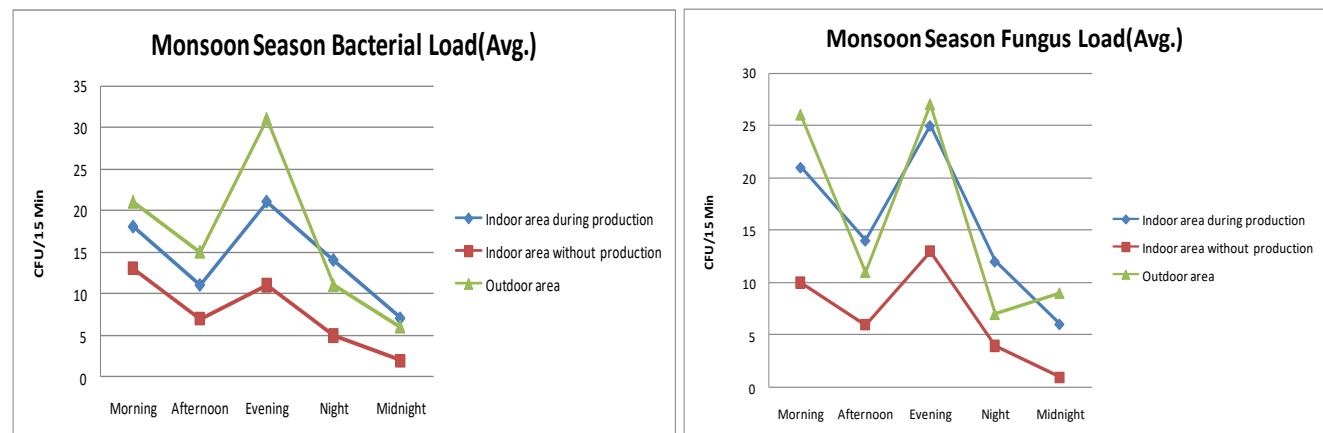
The total bacterial and fungal population observed for different seasons of the year including winter, summer and monsoon at plant level during production and without production as well as at outside the processing plant are given in Table 1, Table 2 and Table 3 respectively. The highest bacterial population recorded in evening stagnant period during 18-20 hrs. at plant level during production and without production is ranging from 11 to 24 cfu compared to morning period during 10 – 11 hrs which is in the range of 10-21 cfu and afternoon period during 12 – 14 hrs, which is ranging from 7 to 17 cfu. While, the bacterial population outside of the plant area during 18 – 20 hrs was the highest in the range of 21 – 44 cfu, as compared to the morning period during 10 – 11 hrs which is ranging from 21 to 47 cfu and afternoon period during 12 – 14 hrs which is ranging from 15 – 36 cfu. The highest fungal population observed at evening stagnant period during 18-20 hrs at plant level during production and without production is ranging from 9 to 25 cfu compared to morning hours during 10 to 11 hrs which is in the range of 6 to 21 cfu and afternoon during 12 – 14 hrs, when the sun rays are normal to the ground which is ranging from 5 to 14 cfu. The highest fungal population observed outside of plant area during evening period during 18 – 20 hrs is ranging from 27 to 49 cfu compared to morning period during 10 -11 hrs which is ranging from 26 to 45 cfu and afternoon period during 12 - 14 hrs which is ranging from 11 to 36 cfu. It was also observed that



**Figure 1**  
Winter Season



**Figure 2**  
Summer Season



**Figure 3**  
Monsoon Season

population of bacterial and fungal flora reduced gradually during night period at plant level ranging from 4 to 9 cfu and 4 to 12 cfu respectively. This was further reduced at midnight at about 12:00 am ranging from 2 to 7 cfu and 1 to 6 cfu respectively.

#### 4. DISCUSSION

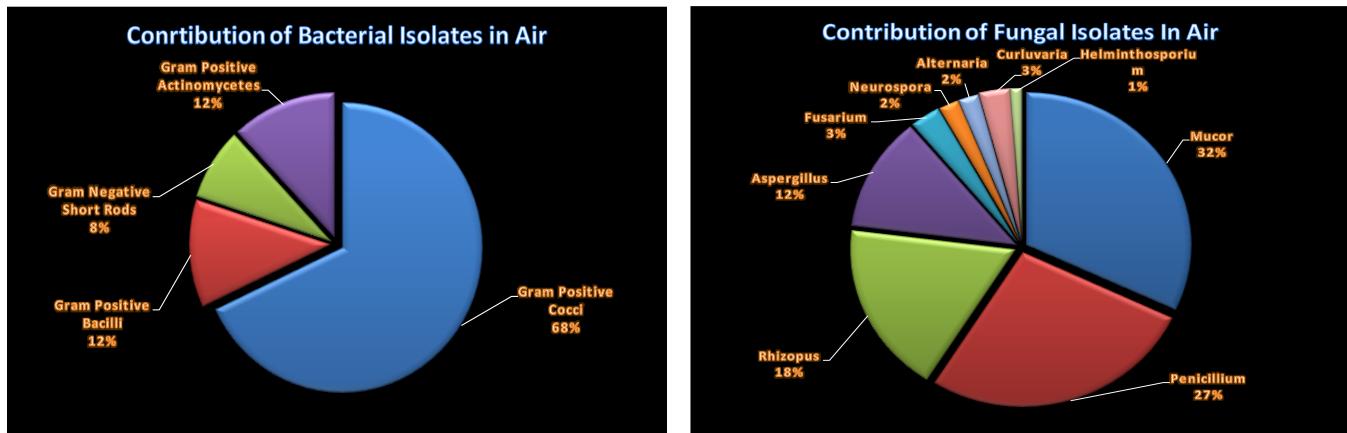
The natural convection or free convection occurs due to temperature difference which affects the density of the air. Lighter air (less dense) with particles will rise, while heavier air (more dense) with particles will fall down; leading to

particle movement. Natural convection will be established due to solar heating of air in a gravitational field.

The air convection current generated due to solar radiation, will lift the particles from the ground surface. Lifting of particles along with microorganisms will depend upon the solar intensity. Solar intensity will increase from morning to noon. After noon, the solar intensity will decrease till evening. Therefore, from morning to noon – there will be lifting of dust particles along with microorganism, which will remain in air at higher level from the ground surface as airborne microorganisms. Because of higher solar intensity

till noon, it will not permit the microorganisms to fall down. Therefore, there will be reduction of microorganisms during noon period at ground levels. However, after noon the solar intensity decreases which will result in decrease of air convection current. Therefore, the microorganisms which have been lifted up under strong air convection current will fall down and increase the microbial load at ground level

of outdoor surrounding microbial population, amount of materials brought from outside such as personal belonging, humidity and temperature of manufactured unit. Human activities such as talking, walking, laughing, coughing, sneezing and sweeping all contribute to the microbial load in air during manufacturing process.



**Figure 7**

Presence of Bacterial and Fungal isolates in air

(Shah et al, 1993). The traffic / vehicle movement will spread and lift the microorganisms from the ground surface. As, during night period there will be gradual reduction in traffic movement till mid night, therefore, there will be gradual reduction in microorganisms at night giving the minimum load at mid night (Fig.1 & 2).

The effect of rain on hourly variation in microbial population is shown in Fig. 3. It can be observed from Fig.3 that the rain causes an immediate reduction in bacterial and fungal population at plant level ranging from 2 to 21 cfu and 1 to 25cfu respectively, while the outside plant area, the bacterial and fungal population is ranging from 6 to 31 cfu and 7 to 27 cfu respectively; which is much less compared to winter and summer seasons of the year. This may be due to wetting and settling of the particles on the ground surface. Air samples were collected 5 times in a day from dairy processing plant and its surrounding at different times of a day. From these samples, four groups of bacteria isolated were identified by Gram staining. It was observed that there was presence of Gram positive *Bacilli* (12%), Gram negative short rods (8%), and Gram positive filamentous *Actinomycetes* (12%). Among fungal isolates at atmospheric and dairy plant levels, *Mucor* (32%), *Penicillium* (27%) *Rhizopus* (18%) and *Aspergillus* (12%) were observed to be most prevailing isolates in the entire unit studied. Least colony numbers were encountered from genera *Fusarium* (3%), *Neurospora* (2%), *Alternaria* (2%), *Curluvaria* (3%) and *Helminthosporium* (1%). This is shown in Fig. 4.

It is observed that within the processing plant, higher microbial counts are recorded during production compared to without production. This could be due to disturbance of ground surface air which leads to the rising of microorganisms from dairy floor due to movement of more people during manufacturing of food products. It is known that when microbial population in air is higher, than it may have more microorganisms and cause to contaminate the dairy products. This may be due to many factors such as the numbers of visitors visiting the manufactured area, transfer

## 5. CONCLUSION

It is evident from the present study that microbial population in air remains higher during evening hours as compared to morning and afternoon period. They were found least during midnight. During morning hours, microbial population was found higher than in the afternoon period. This may be due to establishment of convective air current raising the aerosol particles from the ground/floor surface with rise in sun. At the evening stagnation, these aerosol and soil particles settle on the ground surface causing microbial load in the air at the ground surface. During midnight, microbial count is at lower level, which may be due to settling of aerosol particles on ground surface due to reduction in movement of traffic / vehicles. The air microbial population is found minimum in monsoon season during rain compared to winter and summer seasons. This may be due to maximum settling of aerosols and soil particles at the ground surface. It is also observed that among total bacterial population of air, majority of bacteria was of Gram positive cocci and among of total fungal population, major fungus was of genera *Mucor* and *Penicillium*.

## SUMMARY OF RESEARCH

The study highlights the variation in airborne microorganism during different times of a day, different season of the year, within and outside the dairy processing plant. The knowledge regarding presence of number and types of airborne microorganisms is useful to determine the degree of hygienic condition in dairy processing plant.

## FUTURE ISSUES

From the findings, it deduced that during the afternoon period in any season, microbial flora in the air is least and seasonally the air micro flora found least in the monsoon season, suggesting that the data can be employed for commercial production to boast good quality of manufacturing of dairy or food products.

**DISCLOSURE STATEMENT**

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